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The physical and chemical properties of asbestos fibers which contribute to biological activity

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In spite of the massive experimental work on asbestos toxicity, performed in the past 15 years, the pathogenic mechanism/s at the molecular level are still partially obscure. As recently reported by Kamp and Weitzmann (1999), who did pioneering experiments on asbestos mechanisms, "*…no single mechanism fully accounts for all the complex biological abnormalities caused by asbestos*." This is even more so when it comes to the physico-chemical feature/s which impart the carcinogenic potency to asbestos. ***Particulate toxicants***, i.e. toxicant which act as particles and not as a simple molecule (molecular toxicants), such as asbestos fibres, are complex entities. They act through contact between their surface and cells and tissues. The surface is usually different from the bulk, and exhibits reactive sites, whose nature much depends on the history of the sample. Therefore we may have particles with the same nominal composition, but with remarkably different states of the surface, hence toxicity. Moreover the surface may acquire contaminants from the environment and uptake endogenous molecules when within living matter, hence progressively changing its chemical nature. Because of the long biopersistence, several contacts between fibers and cells may take place in different biological compartments, not necessarily involving the same surface chemical functionalities.

On the basis of experimental finding and epidemiological studies, three main factors appear to contribute to asbestos related health effects: i, the ***form*** of the fiber, ii, the ***mineralogical, chemical and surface composition***, iii, the ***biopersistence*** (Fubini and Otero-Aréan, 1999). These features will be examined separately and analyzed on the basis of the five mechanisms of fiber carcinogenesis reported by Kane et al. (IARC1996).

Long thin fibers are more potent than short ones or isometric analogues. Factor accounting for this are: deposition, easier translocation to the pleura, frustrated phagocytosis, inhibition of clearance, and, in some cases, different surface behavior. Few attempts have been made to investigate which, of the biochemical and cellular responses elicited by asbestos fibers, depend upon fibrous habit and size. Mossman and coworkers have compared fibers with non-fibrous analogues and Davis and coworkers short and long fibers from the same batch. Fibres were more effective than non-fibrous materials, in all the cases examined, except in NO induction (Quinlan et al., 1998); long fibres were always more biologically active than short ones. Some chemical properties, however, were different in short and long fibers (Donaldson et al., 1995; Graham et al., 1999). Surface properties of the non–fibrous analogues were not reported in the relevant papers. More data, from several well characterized sources of materials, are required to discriminate which biological response is related to the physical or to the chemical nature of the fibers.

The composition of the mineral comprises fibrous and non fibrous mineral contaminants, chemical composition of the fiber, state of the surface and surface contamination by exogenous and endogenous matter. As numerous *in vitro* and *in vivo* studies indicate a prominent role of iron catalyzed generation of ROS (Reactive Oxygen Species) and, more recently, RNS (Reactive Nitrogen Species) in the mechanisms of asbestos toxicity (reviewed by Hardy and Aust, 1995; Kamp and Weitzman, 1999), attention needs to be focussed on the iron ions at the fiber surface. Iron may be constitutive of the mineral (crocidolite, amosite, actinolte and antofillite), substitute for magnesium ions (chrysotile, tremolite), or be deposited exogenously/endogenously. The effects caused by iron do not relate with the total iron content (Fubini et al. 1995, Fubini 1996). Iron may be mobilized by chelators or cells (Chao et al, 1994; Chao & Aust, 1994) or be deposited (Shen et al., 2000) Only if iron at the surface is poorly coordinated it may be easily mobilized by endogenous chelators or act at the surface, as a persistent center for the catalysis of ROS. Iron cycling at the fiber surface, as well as a catalytic mechanism of ROS generation, provide chemical systems whose action may continue as long as the

fiber is present, thus accounting for the long latency of asbestos related health effects. Two separate surface sites generate the HO° radical from H_2O_2 or cleave hydrogen-carbon bonds (Fenoglio et al., in press). Iron ions at the solid surface fix irreversibly NO molecules (Martra et al., 1999), suggesting possible interference with iNOS activation. All these effects are modified by thermal or chemical modification of the surface (Otero Arèan et al., 2001; Fenoglio et al., in press). Iron deposition to form asbestos bodies was regarded as defense mechanism towards the fibers. It is a process fiber-selective, as it mostly occurs with long amphiboles fibers. Whether deposited iron is still active is under debate, as contrasting results have been reported (Lund et al., 1994; Ghio et al., 1997). Ferritin, however, was found to adsorb strongly on crocidolite and amosite, be modified and cause significant damage to DNA in presence of ascorbic acid (Fubini et al., 1997; Otero Aréan et al., 1999). This result is in agreement with the increased DNA damage found for amosite-core asbestos bodies, when compared to the effect produced by the naked fiber (Lund et al., 1994).

Biopersistence, is not merely linked to solubility in an aqueous medium. It depends upon deposition, clearing efficiency, which is in turn related to surface properties. *In vivo* extensive reaction with endogenous materials may take place. Both glutathion and ascorbic acid (Brown et al., 2000; Fenoglio et al., 2001), the two major antioxidant defenses in the lung lining layer, have been reported to react with fibers, thus depriving the body of the defenses against the toxic products of the material itself.

At the present level of knowledge any association between each of the mentioned physical and chemical characteristics and the single health outcomes, lung cancer, malignant mesothelioma, fibrosis and pleural plaque, is obviously tentative and speculative. We may note however that the empirical relationships from epidemiological studies report a linear correlation between cumulative exposure and lung cancer, with tobacco smoking acting synergistically. The slope appears industry-specific but the type of asbestos does not seem to be correlated to lung cancer risk (Boffetta et al., 1998) At the opposite, mesothelioma induction is described by a model involving a power function of time since first exposure and latencies from 20 to 40 years, with a carcinogenic potency on the pleura specific to both, industry and type of asbestos (Boffetta et al., 1998). The fiber characteristics causing the processes yielding these two diseases not necessarily should thus be the same: mineral fibers (asbestos, erionite, ceramic fibers) are the only known cause of mesothelioma, suggesting fibrous habit as a perquisite for this type of diseases. The long latency periods indicate a prominent role of biopersistence while a vast number of experimental studies evidence oxidative damage, see e. g. the development of mesothelioma in p53 deficient mice (Marsella et al., 1997) as one of the key event in the development of the disease. As tremolite, erionite and ceramic fibers, which are potent carcinogens for the pleura, do not virtually contain any iron, the oxidative damage will be sustained by few iron traces at the fiber surface, acting as catalysts for ROS generation. Amphiboles, because of their mineralogical structure, are more potent carcinogens than chrysotile. Conversely lung cancer, which is usually associated to persistent inflammation, may be related to the continuos activation of macrophages and PMN by the fiber burden, generating growth factors (fibrosis), inflammatory cytokines, and radicals arising form reactions among fiber derived radical species and cell derived ROS and RNS. Beside deposition and frustrated phagocytosis, any additional role of the fibrous habit in lung cancer still needs to be elucidated. As all asbestos appear nearly equally potent, length and form of the fiber appear non-influent on the outcome of the disease.

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